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ANNEX D

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COMPARATIVE ANALYSIS OF THE SUBGENERA *KERTESZIA* AND *NYSSORHYNCHUS* OF *ANOPHELES* (DIPTERA: CULICIDAE)¹

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ABSTRACT. Lingering questions regarding the current classification of the Neotropical subgenera *Kerteszia* and *Nyssorhynchus* of the genus *Anopheles* are addressed on the basis of comparative morphological data. Homologous characters in the adult, male genitalia, pupa and larva are compared and discussed. The validity of the two subgenera is affirmed using synapomorphic characters. Some interesting characteristics of the immature stages are also considered in terms of ontogeny and heterochrony. The *Myzorhynchella* Section of the subgenus *Nyssorhynchus* is characterized and contrasted with the *Albimanus* and *Argyritarsis* sections for the first time. A brief overview of the history and current status of classification of the genus *Anopheles* and the subgenera *Kerteszia* and *Nyssorhynchus* is presented.

INTRODUCTION

Although it has been said many times that the genus *Anopheles* Meigen is the most studied and best known group of all Culicidae, it cannot be stated that the last word on the classification of the genus or the subfamily Anophelinae has been written. There are numerous questions to be answered regarding the formal status of several subgenera, including the two treated here, and the multitude of informal species groups recognized within each. This is a statement of fact, not intended as criticism of any one cause or published sources. To preface our analysis, we should reflect on the evolution of the confusing and often chaotic taxonomic history of the Anophelinae from about 1901 (the beginning of the influence of Theobald upon mosquito classification) to the present. Perhaps the most dominant forces influencing this evolution have been the inherent and often urgent, medical, economic and political aspects of these studies on this most important of mosquito

vector groups. We believe that studies on the basic concepts and classification of the Anophelinae would have benefited significantly from the absence of one or more of these forces.

Most of the more significant taxonomic studies on the genus *Anopheles* during the past 50-60 years were by investigators that were first malariologists or epidemiologists, and secondarily taxonomists, who were most often, by necessity, concerned with the description or elucidation of individual species in connection with, or in support of, studies on disease vector relationships. Except for a few comprehensive regional studies on selected infrasubgeneric species groups, e.g., Reid (1950, 1953, 1962, 1965), Colless (1956), Harrison (1980), Faran (1980) and Linthicum (1988), few can be viewed as true taxonomic revisions. They are, at best, reviews, and a few are mere compilations of previous works. Few investigators, if any, enjoyed the luxury of time, resources or political freedom needed to delve into the more complex phylogenetic aspects of the formal supraspecific groups. As Belkin (1962) stated: "Unfortunately, practically all the studies have been local or regional in scope, and no attempt has been made to date to monograph the genus from a world standpoint." As a direct consequence of the

¹ The views of the authors do not purport to reflect the views of the Department of the Army or the Department of Defense.

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early works of Theobald, when many new genera were created based in large part on the character of individual scales and the placement of these scales, the identification and placement of known or new species into the proper groups became increasingly difficult or impossible. As additional new species were being discovered at a rather rapid pace, chaos ensued. It soon became apparent that a system of classification based on Theobald's characters was neither practical nor natural. One of the early critics of Theobald's classification was Knab (1913a) who stated: "the subject was made needlessly difficult by hasty work and by the sub-division of the old genus *Anopheles* into numerous ill-defined and fancifully differentiated genera. The intricacies of this 'system,' unwarranted from both a scientific and practical standpoint, even the trained entomologist could not tread with safety, and to others it could be no less than hopeless or disastrous." Consequently, between 1915 and 1932 there was a near complete abandonment of the Theobald classification of *Anopheles* with a concomitant move toward a much more conservative system for the Anophelinae, from about 37 genus-group names to the current three. A similar move occurred with subgenera, to arrive at the current six for the genus *Anopheles*.

The current system of subgeneric classification for *Anopheles* can be credited to Christophers (1915), even though he did not propose it to be the long-term solution to classification. He stated: "As my own material (40 species) supplemented by the valuable drawings of Howard, Dyar and Knab [1912] (15 species) has enabled me to study the genitalia of practically all the important groups of species in the subfamily, I have felt justified in making some generalizations regarding the male genitalia in *Anopheles* and their use in the classification of the group. The genitalia would appear to offer the most satisfactory means yet put forward for this latter purpose." In concluding the review of the genitalia for all species or groups studied, he stated: "Though I am not at present concerned with any discussion upon nomenclature, it may be pointed out that, so far as the male genitalia are concerned, generic subdivision of the Anophelini would very naturally take the follow-

ing form . . ." This is followed by a table with his three subgenera: *Anopheles*, *Myzomyia* Blanchard (= *Cellia* Theobald) and *Nyssorhynchus* Blanchard, accompanied by 10 synonymous Theobald genus-group names. There was no mention of *Kerteszia* Theobald or *Myzorhynchella* Theobald, although the species *An. bellator* Dyar and Knab (presently a member of the subgenus *Kerteszia*) was associated with *Nyssorhynchus*. The primary genitalic characters used in this classification scheme were the number and placement of specialized setae on the gonocoxite, currently referred to in Faran (1980) as the parabasal, accessory and internal spines. Edwards (1932) and Christophers (1933) formally adopted this system of classification for the *Anopheles*, adding the Neotropical subgenus *Stethomyia* Theobald to the list and formally recognizing the genera *Bironella* Theobald and *Chagasia* Cruz. These two authors included three species groups in the subgenus *Nyssorhynchus*: *Nyssorhynchus*, *Myzorhynchella* and *Kerteszia*. Little new information has been added to this basic characterization of the subgenera of *Anopheles*, although considerable shifting in and out of synonymy of several subgenera and species groups has occurred since.

Several significant works on *Anopheles* have been published since 1933, and all cite Edwards (1932), Christophers (1933) or both as their authority on classification, although a few expressed uncertainties. Belkin (1962), in provisionally following the system of Edwards (1932), stated: "There is little agreement at present regarding the internal classification of the genus, although at least 4 subgenera are uniformly recognized: *Stethomyia* (Neotropical), *Anopheles* (widely distributed), *Nyssorhynchus* (Neotropical), and *Cellia* (Old World). In the Neotropical fauna, recent workers have recognized several additional subgenera. It is evident that in the American Mediterranean Region there are several annectant groups. Until these are thoroughly studied and evaluated, there is little basis for a natural classification of the genus. For the present I am following the system of Edwards (1932), as modified by subsequent workers, for the Old World fauna."

We hope that the present thorough comparative morphological study of the subgenera

Kerteszia and *Nyssorhynchus* will be a significant step toward an eventual natural classification of the genus *Anopheles*, and that it will serve as a foundation for future comparisons with other groups. The recent paper by Wilkerson and Peyton (1990) was also a significant step in the direction of a natural classification of *Anopheles*. They introduced numerous new credible homologies for wing spots which provided insight into the classification problems, and these have had a strong influence on the development of this paper. A sound, coherent, natural system of classification based on the principles of comparative morphology, using homologous, synapomorphic characters embracing all life stages, provides for stability, not chaos, and it ultimately makes the identification of individual species much easier.

Kerteszia and *Nyssorhynchus* are currently recognized as subgenera of the genus *Anopheles*. Both include several medically important species, and each was originally described as a genus. Data are presented in Table 1 which unequivocally demonstrate that the two are well-defined monophyletic groups deserving subgeneric, if not generic, status. We do not propose changes in their status, for this would require a thorough comparison with the other subgenera, which is beyond the scope of this paper. *Nyssorhynchus*, a replacement name for *Lavernia* Theobald, contains 27 valid species subdivided into three somewhat incompletely defined informal species groups designated as the Albimanus Section (15 species) (Faran 1980), the Argyritarsis Section (8 species) (Linthicum 1988) and the Myzorhynchella Section (4 species). The latter was originally described as a genus by Theobald (1907), characterized as a subgenus by Galvão (1941) and recently considered a poorly known species group of uncertain taxonomic status (Faran 1980, Linthicum 1988). Here, for the first time, we confirm this group as a natural assemblage of species within the subgenus *Nyssorhynchus*, possessing all of the significant morphological characteristics of the other better known members of the subgenus. We recognize it as a section, coequal in status with the two better known sections. *Kerteszia* currently contains 12 species without subdivisions. Although originally

described as a genus, this group has not enjoyed the relative stability of *Nyssorhynchus*. It has been in and out of synonymy with *Nyssorhynchus* numerous times, most often as a species group, but sometimes even going unmentioned. Dyar and Knab (1918) confused the status of *Kerteszia* by proposing a new subgenus for *An. cruzii* Dyar and Knab (presently in the subgenus *Kerteszia*) and emphatically stating that *Kerteszia* "evidently cannot be used as a subgeneric name for the bromelicolous species."

Zavortink (1973) was the last reviewer of the subgenus *Kerteszia*. He stated: "The bromelicolous species of *Anopheles* have been recognized as a distinct group since the works of Knab (1913[b]:15-17) and Dyar and Knab (1917:38-40) and their relationship to *Nyssorhynchus* has been known since the early studies of Root (1922a:322; 1922b:388; 1923:271, 277-278). Edwards (1932:44,46), following Christophers (1924:42), included *Kerteszia* in the subgenus *Nyssorhynchus*, but Dyar (1928:467-470) and Komp (1937:492-529; 1942:8-9, 162-165) treated it as a distinct subgenus. For the time being I am following the currently accepted classification, as reflected in Stone, Knight and Starcke (1959), and am recognizing this monophyletic group as a distinct subgenus of *Anopheles*. However, the relationship of *Kerteszia* to *Nyssorhynchus* may prove to be so close that the former will again be recognized as only a species group of the latter."

Komp (1942) studied the *Anopheles* of the Caribbean Region and pointed out 15 differences in the adult females, male genitalia and larvae of *Kerteszia* and *Nyssorhynchus*, and stated he believed "the 'group' *Kerteszia* of Edwards' classification should be elevated to a subgenus, coequal with subgenus *Nyssorhynchus*." Zavortink (1973), however, felt that these differences may not be indicative of separate subgeneric status. This provided the impetus for us to look for subgeneric differences while conducting detailed studies on malaria vectors and related species within these groups. This study has led to the discovery of at least 57 diagnostic differences between species of *Kerteszia* and *Nyssorhynchus*. These morphological distinctions are the subject of this paper.

Table 1. Comparison of *Anopheles* subgenera *Kerteszia* and *Nyssorhynchus*.

| Life stages and morphological features | <i>Kerteszia</i> | <i>Nyssorhynchus</i> |
|--|--|---|
| Adults (Figs. 1-3) | | |
| <i>Thorax</i> (Fig. 1): | | |
| Scutellum | Without scales (rarely with a few dark and/or white scales medially) | With numerous, scattered white, yellow or silver scales along posterior border |
| Scutal scales | With a few scales in patches on lateral and/or anterior areas | With numerous scales also on acrostichal and dorsocentral areas |
| Scutal integument | With 4 distinct dark, pollinose, longitudinal lines | Without distinct dark longitudinal lines |
| <i>Legs</i> (Fig. 1): | | |
| Hindfemur | With a long white stripe on distal 0.33 anteriorly | With a small isolated pale spot on distal 0.25 anteriorly |
| Midfemur | With a moderately narrow white stripe on distal 0.33 anteriorly | With a small isolated pale spot on distal 0.22 anteriorly and a small dorsoapical spot |
| <i>Wing</i> (Fig. 2): | | |
| Position of crossveins sc-r and r ₁ -r ₅ | Far apart, distance about 0.5 length of sector dark spot (SD) | Close together, about 0.30 length of sector dark (SD) or less |
| Vein R at humeral crossvein (h) (dorsal) | With small dark-scaled spot | Without dark-scaled spot |
| Subcostal vein (Sc) | Ends before subcostal pale spot (SCP), varies from near 0.75 of sector dark spot (SD) to distal end of sector dark spot | Ends at proximal end of subcostal pale spot (SCP) or near middle of subcostal pale spot |
| Vein R ₄₊₅ | Dark-scaled apically, usually ending in dark fringe, if ending in pale fringe spot then spot not isolated, involving end of vein R ₃ also | Narrowly pale-scaled apically, with corresponding isolated apical pale fringe spot |
| Vein CuA | Mostly dark-scaled, with basal 0.12 or more dark | Mostly pale or about equally pale and dark, without basal dark spot |
| Vein 1A | All dark or rarely with 1 or 2 very small pale spots | With 3 pale-scaled areas setting off 2 dark areas between a basal and an apical pale spot (greater amount of dark scales in <i>Myzorhynchella</i>) |
| <i>Male genitalia</i> (Fig. 3): | | |
| Aedeagus | Slender, uniformly tapered from base to very narrow rounded apex | Broad, not noticeably tapered, at least on basal 0.8 |
| Subapical seta | Absent | Present |
| Accessory setae | 2 subequal setae inserted beyond basal 0.5 (0.6-0.7 in Zavortink 1973) (tergomesal) | 2 subequal setae inserted at about proximal 0.4 (slightly more tergal) |
| Internal seta (position) | Far basad of accessory setae (0.4-0.5 from base in Zavortink) | Closely associated with and at least slightly caudad of accessory setae |
| Internal seta (development) | Very stout, equal to accessory seta in development, end acute or blunt | About as long as accessory setae but much weaker, acuminate |

Table 1. Continued.

| Life stages and morphological features | <i>Kerteszia</i> | <i>Nyssorhynchus</i> |
|--|---|--|
| Parabasal seta | Long, considerably longer than accessory setae or internal seta (about 1.5) (tergal) | Shorter, about 0.6 or less length accessory setae |
| Ventral lobes of claspette | Independent, narrowly joined basoventrally | Fused to form a single median structure |
| Dorsal lobes of claspette | Each with 2 distinct groups of 3-5 long, apically flattened setae | Each with a single group of 2-3 apically flattened setae |
| Pupae (Figs. 4,5) | | |
| <i>Cephalothorax</i> (Fig. 4): | | |
| Pinna of trumpet | Short, less than 0.5 of trumpet length | Long, greater than 0.5 of trumpet length |
| Meatal cleft (slit) of trumpet | Absent | Present |
| <i>Abdomen</i> (Fig. 5): | | |
| Seta 9-I | Minute, single, much shorter than 6-7-I (less than 0.3) | Long, single, 0.5-1.0 length of 6-I, longer than 7-I |
| Seta 1-II,III | Weak, moderately short, single or with a few distal branches | Well developed, relatively long, multibranched |
| Seta 14-III | Absent | Present |
| Seta 1-IV-VII | Short, weak, less than 0.5 of segment, usually branched | Long, well developed, greater than length of segment, single (rarely one or more double) |
| Seta 2-IV,V | Cephalad of and nearly on line with 3-IV,V considerably laterad of 1-IV,V, sometimes laterad of 3-V | Conspicuously mesad of 3-IV,V and sometimes mesad of 1-IV,V |
| Seta 5-V-VII | Short, weak, usually with distal branching, less than 0.5 length of segment | Long, well developed, single, at least 0.7 length of segment, usually longer |
| Seta 3-VI | Laterad of 1-VI (determined by innervation on prepupal larva) | Mesad of or on line with 1-VI |
| Seta 10-VI | Present | Absent |
| Base of paddle | With distinct darkly pigmented line (weakly developed in <i>An. bambusicolus</i>) | Without darkly pigmented line |
| Seta 1-P | Short, stout, shorter than 2-P (2-P absent in <i>An. neivai</i>) | Longer, slender, similar to or longer than 2-P or long sinuous and hooked apically |
| Larvae (Figs. 6-9) | | |
| <i>Head</i> (Figs. 6,7): | | |
| Seta 1-A | Single, simple, inserted dorso-laterally | Branched (2-10), inserted dorsomesally |
| Seta 2-C | Inserted on same level or slightly posterior to 3-C | Inserted anterior to 3-C |
| Seta 4-C | Inserted posterolateral to 3-C, unbranched or with a few aciculae apically | Inserted posteromesal to 3-C, single or branched |
| Setae 5-7-C | Single, simple or sparsely aciculate | Plumose |
| Seta 11-C | Long, single, simple or with few terminal irregular branches or aciculae | Strongly plumose from base |
| Hypostomal suture | Undeveloped or very short, in-apparent | Well-developed, but not reaching tentorial pit |

Table 1 continues.

Table 1. Continued.

| Life stages and morphological features | <i>Kerteszia</i> | <i>Nyssorhynchus</i> |
|--|---|--|
| <i>Thorax</i> (Fig. 8): | | |
| Seta 1-P | More or less developed as a weak central stem with short weak lateral branches or aciculae | Palmate, plumose or fanlike, often with leaflets |
| Seta 2-P | Not borne on prominent sclerotized tubercle | Borne on prominent sclerotized tubercle with 1-P |
| Setae 9- and/or 10-P, M, T | Lightly to strongly aciculate | Simple or single (9-P, T branched on <i>An. albimanus</i> only) |
| Seta 14-P | 2 or 3 branches | 4 or more branches (4-15) |
| Seta 4-M | Moderately long to long, nearly equal to 6-M in length and usually significantly longer than 2-M | Short, not more than 0.5 as long as 6-M and usually about as long as 2-M |
| Seta 8-M, T | Weakly plumose, lateral branches very short | Strongly plumose, branches long |
| Seta 3-T | Long, weak central stem with sparse lateral branches or aciculae | Palmate or fanlike, usually with leaflets |
| <i>Abdomen</i> (Figs. 8, 9): | | |
| Seta 2-I-VI | Single, simple (one or 2 may be aciculate in <i>An. homunculus</i> , <i>An. cruzii</i>) | Multibranched on I-III and VI, single on IV, single or branched on V |
| Setae 6, 7-I, II | Weakly plumose, lateral branches short | Strongly plumose, branches long |
| Seta 6-III | Aciculate, differing markedly from 6-I, II in branching and degree of development, similar to 6-IV, V | Plumose, similar to 6-I, II in branching and development |
| Seta 14-III | Absent | Present |
| Seta 2-IV (development) | Single, very long, about as long as segment or more and longer than 2-V | Short, less than 0.6 length of segment and considerably shorter than 2-V |
| Seta 2-IV (position) | Laterad of 4-IV | Mesad of 4-IV |
| Seta 6-IV-VI | Single, aciculate or 6-VI occasionally with a few longer basolateral branchlike aciculae | Single, simple or branched (branched in <i>Myzorrhynchella</i>) |
| Setae. 2, 3, 5-VIII | Single, simple or with few lateral branches or weak terminal branches, sometimes aciculate | Well-developed, multi-branched |
| Seta 1-S | Weak, single or with 2-3 branches or weak terminal branches or aciculae | Well developed, multi-branched |
| Seta 2-S | Minute, single | Small, multibranched |
| Seta 8, 9-S | Weak, single (8-S absent in <i>An. neivai</i>) | Branched (9-S single or double in <i>An. darlingi</i>) |
| Seta 4-X | With 9 pairs of setae | With 8 pairs of setae |
| Pecten spines | Uniform length or uniformly alternating long and short in middle of row | Neither condition; with 2-7 spines conspicuously longer than others, randomly placed, except for a long one at each end of row |

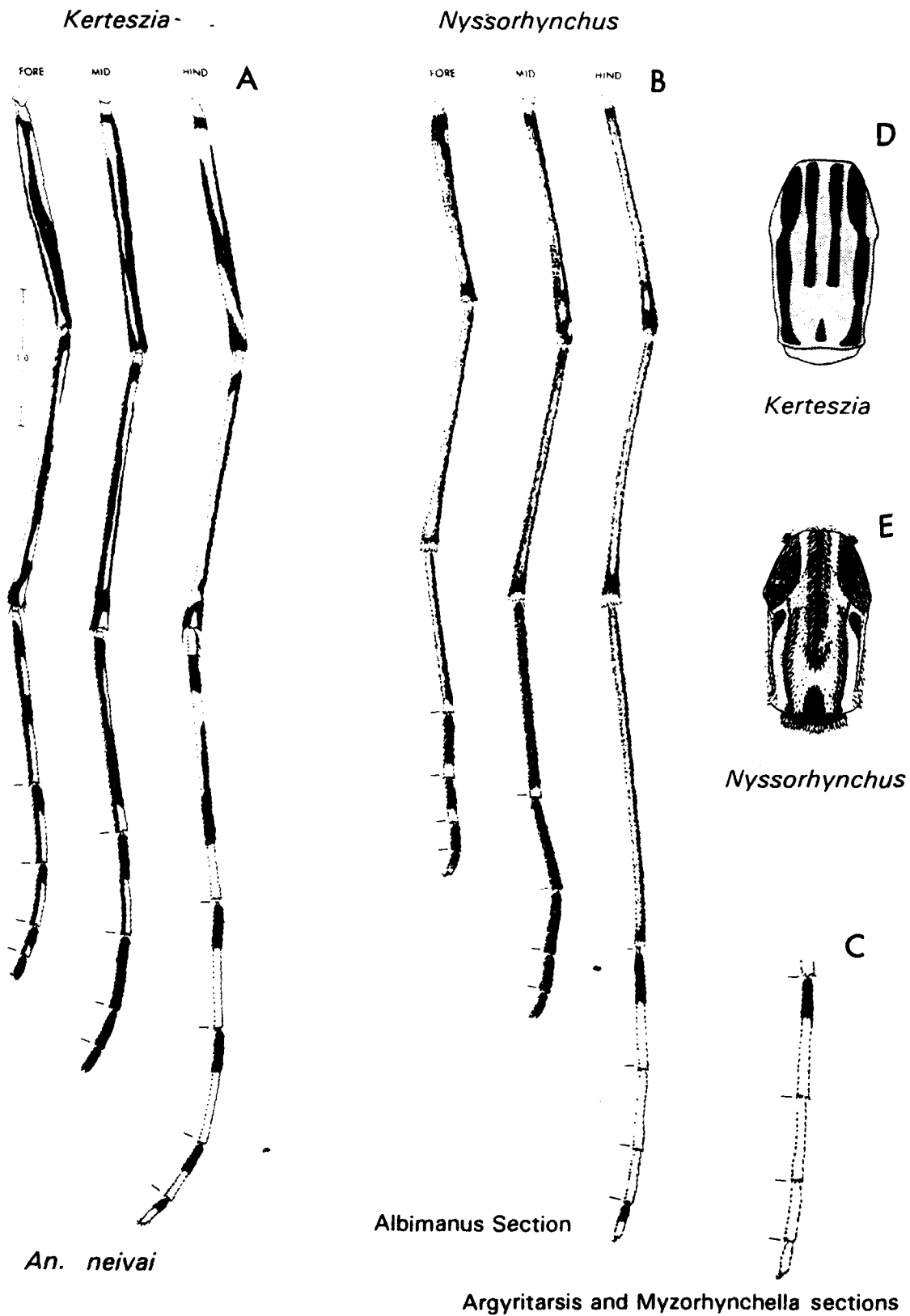


Fig. 1. A,D. From Zavortink 1973; B,E. From Faran 1980; C. From Linthicum 1988.

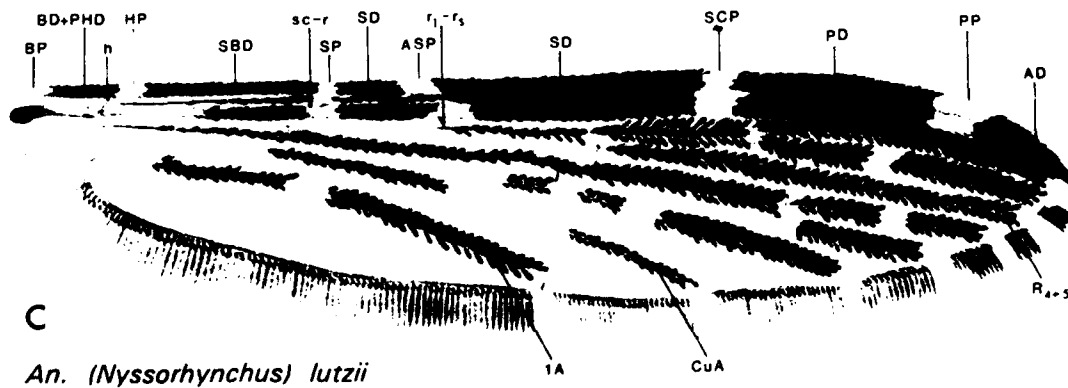
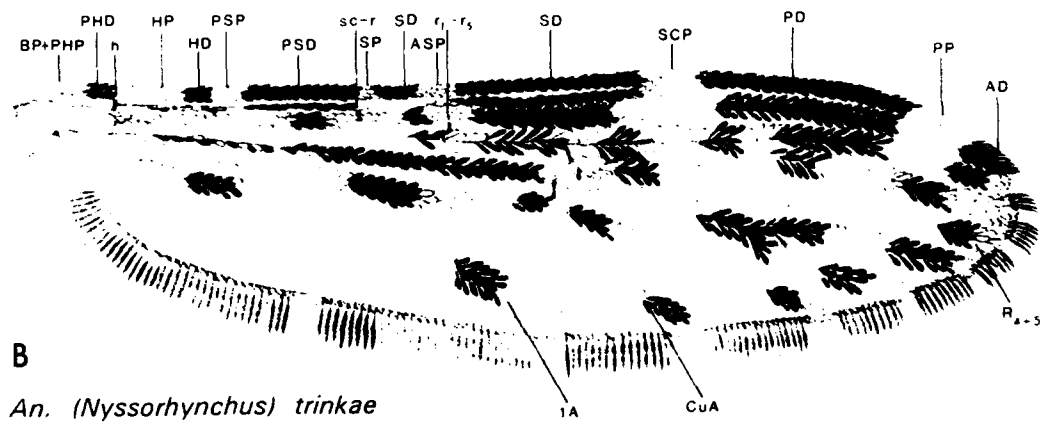
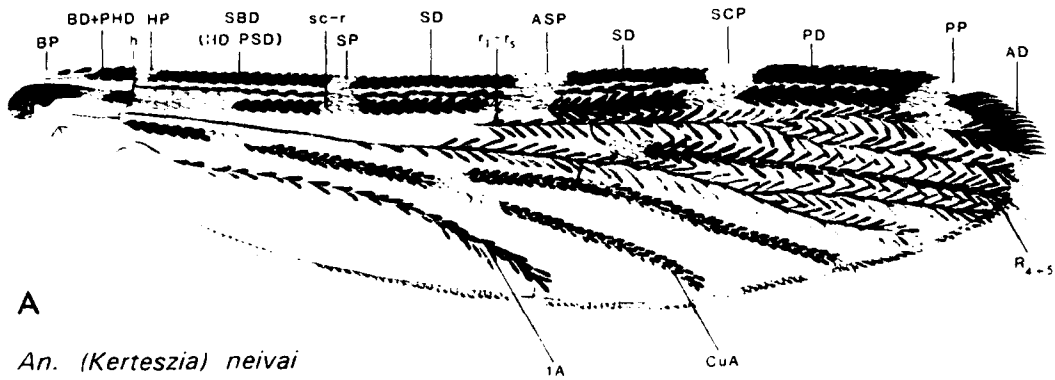


Fig. 2. A,B. From Wilkerson and Peyton 1990; C. Original.

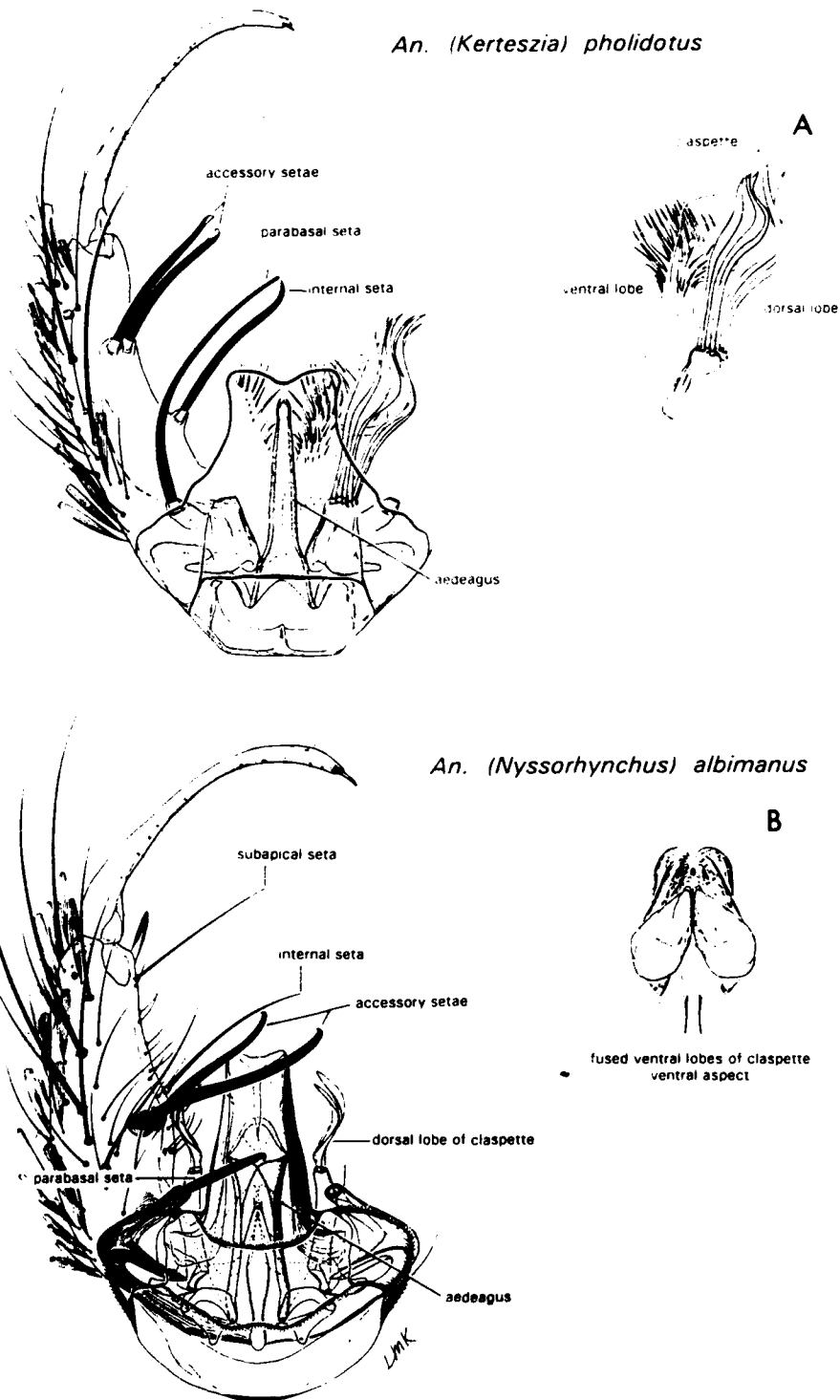


Fig. 3. A. From Zavortink 1973; B. From Faran 1980.

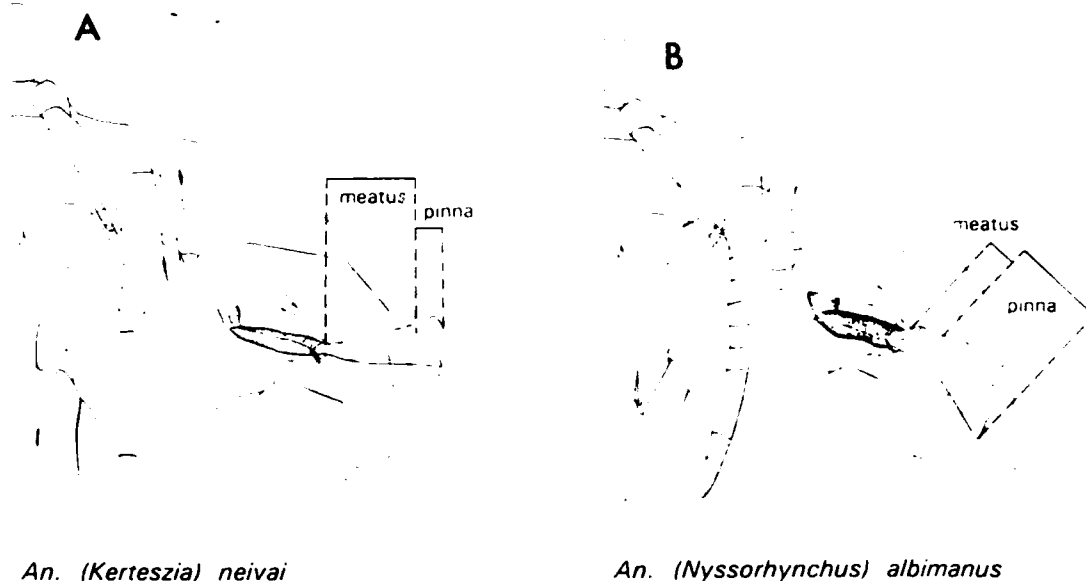


Fig. 4. A. From Zavortink 1973; B. From Faran 1980.

MATERIALS AND METHODS

The search for and study of morphological differences between *Kerteszia* and *Nyssorhynchus* involved a combination of examining specimens (where available) and examining descriptions and illustrations in several published works. It was not always possible to rely upon the illustrations in many of the early works on *Anopheles*, because of obvious inaccuracies or incomplete presentation of some characters or life stages. The most valuable references with very accurate and complete illustrations of all life stages (except the egg) of the species treated were Zavortink (1973), Faran (1980) and Linthicum (1988). We have drawn heavily from these works. Other pertinent references with useful illustrations were Galvão (1941), Rachou and de Sousa (1950) and Faran and Linthicum (1981). All of the significant, diagnostic characters determined to be consistently different in all members of the two subgenera are contrasted in Table 1. We define 57 subgeneric character differences distributed as follows: adults (12), male genitalia (8), pupae (12) and larvae (25). The actual number of individual characters is greater (86), but we have, in several cases, chosen to combine two or more independent morphological elements, e.g.,

larval setae 5-7-C, 6-IV-VI, pupal seta 5-V-VII, etc. The listing of characters for the immature stages is not exhaustive. There appear to be other less apparent characters, such as relative lengths of some setae, particularly on the larval head, but we hesitate to include these without further study of the range of variation and feel that the inclusion of these would not add significantly to our case. In the case of larval and pupal setae, we have focused more on the overall characteristics of each and less on slight differences in length, except where the differences are unequivocal. The characters in Table 1 are self-explanatory and can be compared in examples of the two subgenera presented in Figs. 1-9. The examples are photostatic reproductions (with slight alterations, mostly labeling) of figures appearing in Belkin (1962), Faran (1980), Floore et al. (1976), Linthicum (1988), Wilkerson and Peyton (1990) and Zavortink (1973). The adult wing of *An. (Nys.) lutzii* Cruz (Fig. 2), head of first instar larva of *An. (Nys.) albitarsis* Lynch Arribalzaga (Fig. 6) and larval pecten plate of *An. (Nys.) albimanus* Wiedemann (Fig. 9) are originals. In addition, selected characters of an unusual nature or of ontogenetic significance are discussed below.

Table 2 presents known characters for distinguishing the three informal infrasubgeneric

An. (Kerteszia) neivai

An. (Nyssorhynchus) albimanus

A

B

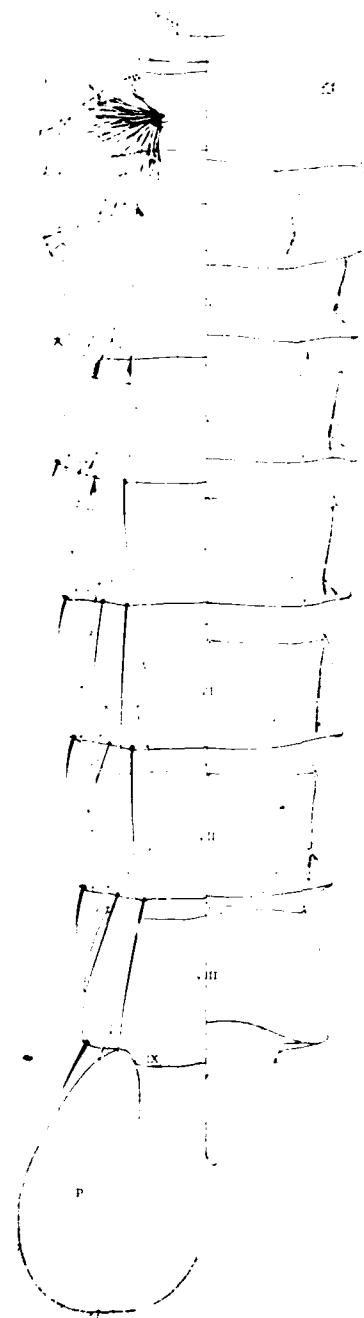
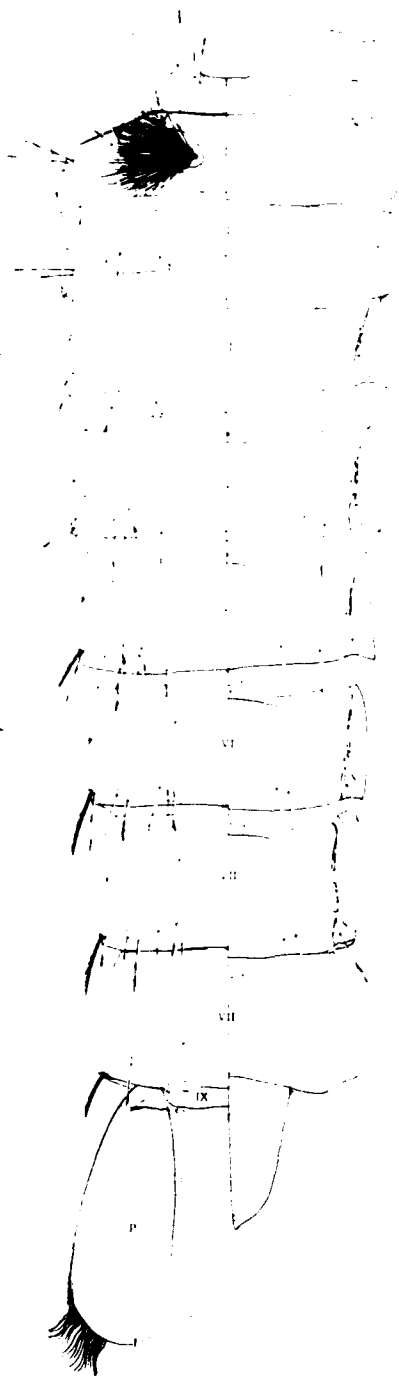
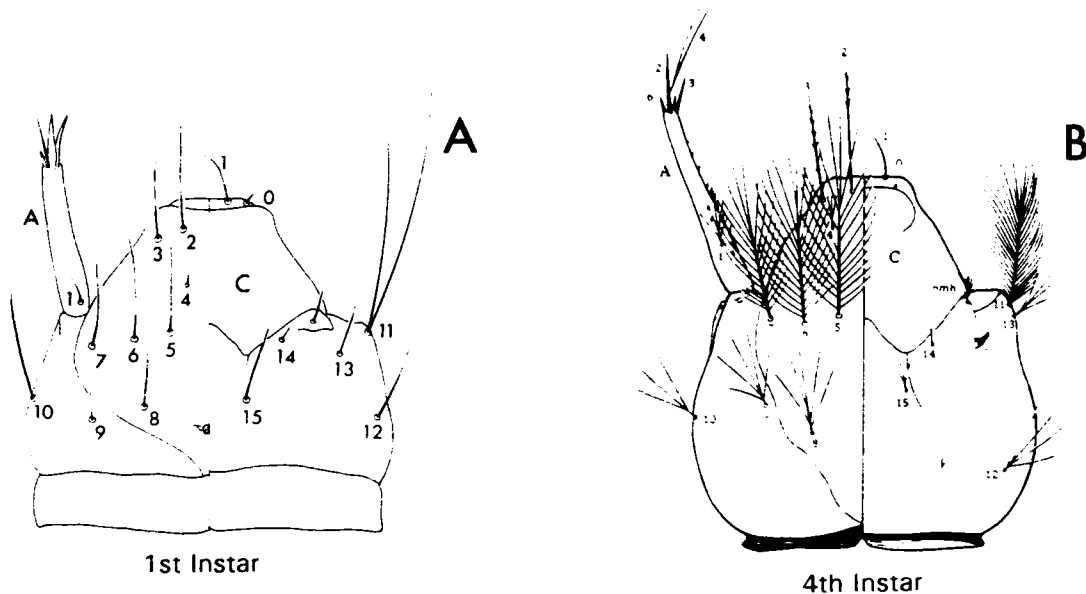


Fig. 5. A. From Zavortink 1973; B. From Faran 1980.

An. (Nyssorhynchus) albitarsis



species groups of the subgenus *Nyssorhynchus*. Some of the characters are more differential than diagnostic, i.e., the characters may overlap. Nevertheless, when used in combination with diagnostic characters, these further characterize the groups.

The morphological terminology and numbering of larval and pupal setae follow Harchach and Knight (1980, 1982). Wilkerson and Peyton (1990) are followed for wing spot nomenclature.

DISCUSSION

Although the morphological differences between the two subgenera are adequately defined in Table 1, a few characters deserve additional discussion. These are regarded as having considerable phylogenetic significance. They include possibly unique or unusual characters, and others of ontogenetic significance.

Adults (Figs. 1-3). *Legs* (Tables 1,2): There is a general perception that *Nyssorhynchus* and *Kerteszia* can be characterized on the basis of the distribution of pale scales on the hindtarsus. In general, species of *Nyssorhynchus* and *Kerteszia* can be separated on the

An. (Kerteszia) neivai

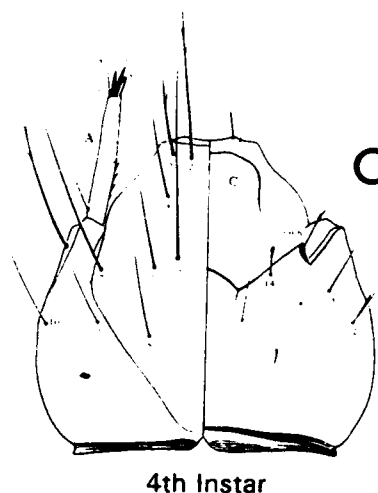
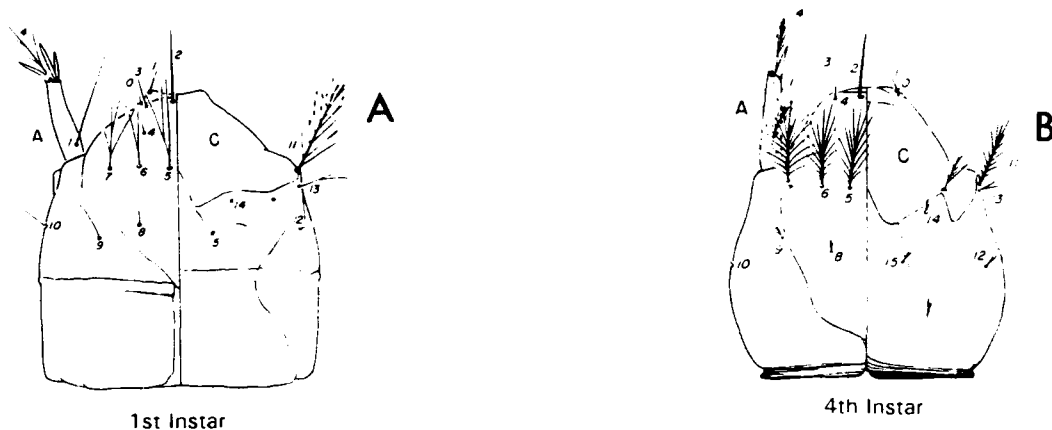


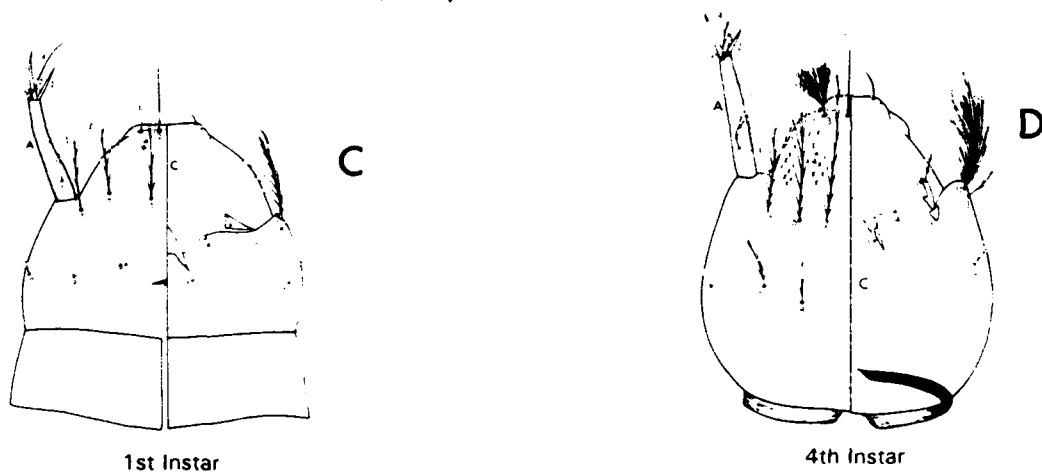
Fig. 6. A. Original; B. From Linthicum 1988; C. From Zavortink 1973.

common condition of dark basal bands on hindtarsomeres 3 and 4 in *Kerteszia*, and these tarsomeres all white in *Nyssorhynchus*. However, two uncommonly encountered species of *Nyssorhynchus*, *An. rondoni* (Neiva and Pinto), Albimanus Section, and *An. nigritarsis* (Chagas), Myzorhynchella Section,

Bi. (Brugella) hollandi



An. (Anopheles) crucians



An. (Cellia) punctulatus

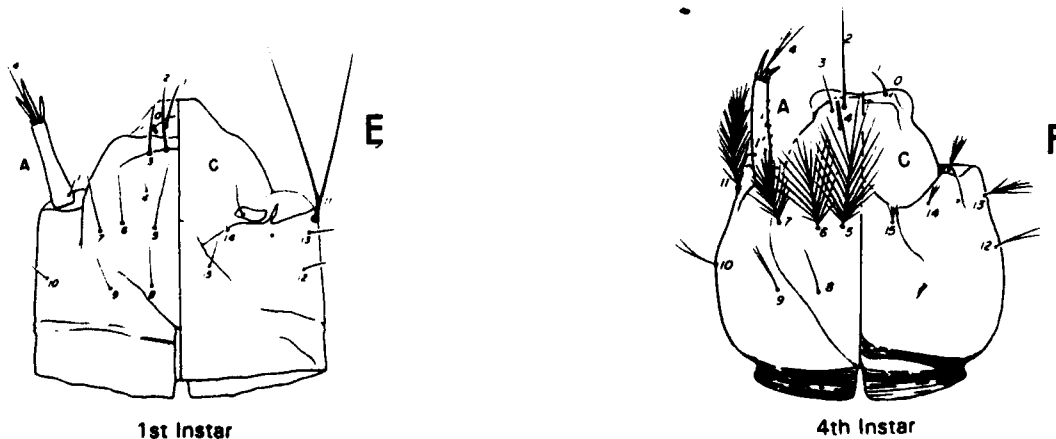


Fig. 7. A,B. From Belkin 1962; C,D. From Floore et al. 1976; E,F. From Belkin 1962.

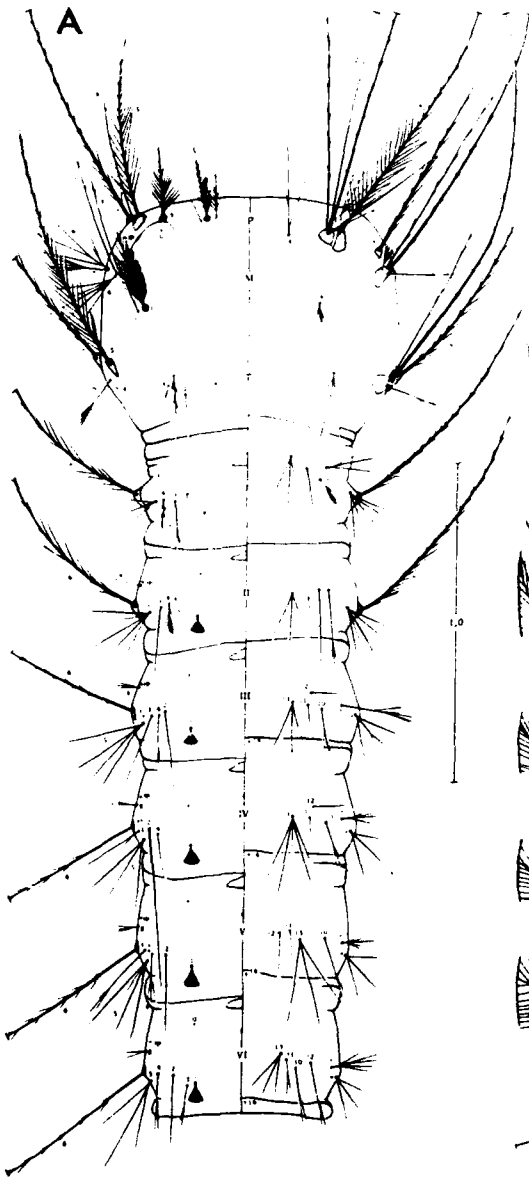
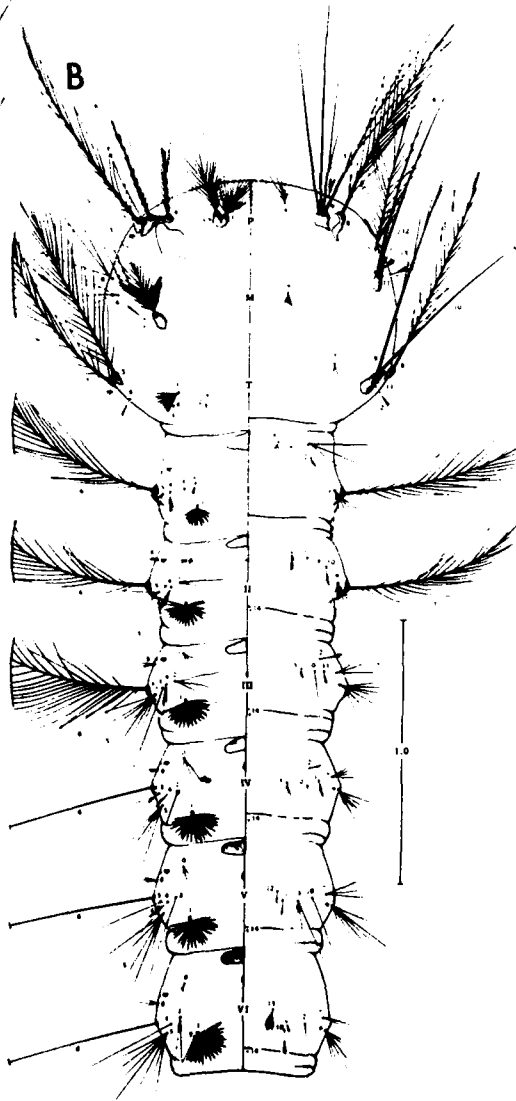
An. (Kerteszia) neivai*An. (Nyssorhynchus) albimanus*

Fig. 8. A, From Zavortink 1973; B, From Faran 1980.

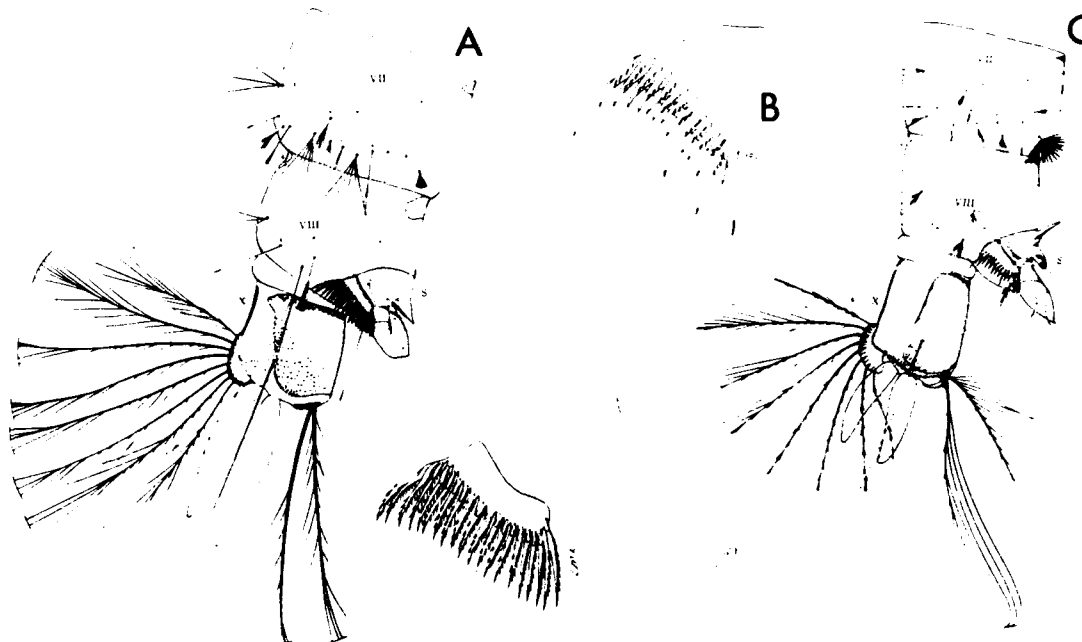
*An. (Kerteszia) neivai**An. (Nyssorhynchus) albimanus*

Fig. 9. A. From Zavortink 1973; B. Original; C. From Faran 1980.

are exceptions and invalidate this as a subgeneric distinction. The significant subgeneric difference in leg markings is the subapical pale markings on the anterior surface of the mid- and hindfemora. The small isolated spots that

are present in all three sections of *Nyssorhynchus* appear to be diagnostic, as we are unaware of any other *Anopheles* quite like this. In contrast *Kerteszia* have complete or broken longitudinal lines or streaks on these femora.

Table 2. Comparison of the three sections of the subgenus *Nyssorhynchus*.

| Life stages and morphological features | Albimanus Section | Argyritarsis Section | Myzorhynchella Section |
|--|---|---|---|
| Adults | | | |
| Small caudolateral patches of dark scales on abdominal terga III–VII | Present | Present, except for <i>An. pictipennis</i> . | Absent |
| Basal dark band on hindtarsomere 5 | Present | Absent | Absent |
| Hindtarsomeres 3,4 | All white, except 3 dark on basal 0.2–0.35 of <i>An. rondoni</i> | All white | All white, except basally dark on 3 and 4 of <i>An. nigratarsis</i> |
| Vein 1A | Mostly pale-scaled, with long median pale area and 2 short dark areas | Mostly pale-scaled, with long median pale area and 2 short dark areas | Mostly dark-scaled, with short median pale area and 2 long dark areas |
| Pupae | | | |
| Seta 0–II–V | Small, well developed, branched | Small, well developed, branched | Minute, single (confirmed for <i>An. parvus</i> only) |
| Larvae | | | |
| Seta 6–IV–VI | Single | Single | Branched |

Wing (Table 1, Fig. 2): The long distance between the positions of crossveins sc-r and r₁-r₅ in *Kerteszia* is a highly derived, apparently unique character within *Anopheles* and appears to be unique within the Culicidae. Genera have been established on lesser distinctions in the past and we believe that this character alone could justify the generic separation of *Kerteszia* from *Nyssorhynchus* and other subgenera if confirmed to be unique. Since the sector pale (SP) and accessory sector pale (ASP) spots are consistently associated with these crossveins (Wilkerson and Peyton 1990), they are correspondingly far apart in *Kerteszia*, placing the ASP in an unusual position at the middle of the sector dark spot (SD). The position of these crossveins shown for *Nyssorhynchus* in Fig. 2 is somewhat typical for all other *Anopheles*.

The small dorsal patch of dark scales on vein R at the humeral crossvein (h) in *Kerteszia* is characteristic of the group and completely absent in *Nyssorhynchus*. Galvão (1941: Fig. 20) illustrated the wing of *An. parvus* (Chagas) of the Myzorrhynchella Section with a small dark-scaled spot on vein R at the humeral crossvein. This apparently is an error, for we have examined several specimens of *An. parvus* without finding any evidence of dark scales in this region of the wing.

Male genitalia (Table 1, Fig. 3): In addition to the obvious differences in position of the accessory setae and the degree of development of the parabasal, accessory and internal setae of the two subgenera, there is no comparable setal development in other subgenera of *Anopheles*. At a casual glance, these setae appear to be very similar in the two subgenera, which may have influenced the earlier treatment of *Kerteszia* as a synonym of *Nyssorhynchus*.

Pupae (Table 1, Figs. 4,5). *Seta 9-I*: The development of this seta in *Kerteszia* is the normal condition encountered in the majority of *Anopheles* species. This seta is unusual in *Nyssorhynchus*, and stands in striking contrast to that of *Kerteszia*. Harrison (1980) reported a similarly developed seta 9-I in the Old World Pyrethrophorus Section of the subgenus *Cellia*, which includes the Gambiae Complex.

Seta 14-III: The absence of this seta in *Kerteszia* is a significant ontogenetic distinction. We are not aware of its absence in any other group of *Anopheles*. Of added significance is its absence in fourth instar larvae, and possibly earlier instars.

Seta 3-VI: The position of this seta is an ontogenetic distinction which was not possible to demonstrate without confirmation of innervation in late prepupal-stage larvae. We examined two specimens of *An. (Ker.) cruzii* that clearly show the nerve connections between setae of the fourth stage larva and the developing pupa. These specimens show that the developing seta 3-VI of the pupa is placed laterad of seta 1-VI, a rare contradistinction to its usual mesal position in the majority of *Anopheles*. This condition is also known in the genus *Bironella* (Belkin 1962, Tenorio 1977) and in the Umbrosus Group of the subgenus *Anopheles* (Harrison and Scanlon 1975). Although the position of seta 3-VI is confirmed in only *An. cruzii*, we believe this condition probably exists in all species of *Kerteszia*.

Seta 10-VI: The failure of this seta to be carried over from the larva to the pupal stage is known to us only in *Nyssorhynchus* and is an excellent diagnostic character. Its absence is easily noted and, because there is no alveolus, specimens that have the seta broken off cannot be confused for species of *Nyssorhynchus*.

Larvae (Table 1, Figs. 6-9). *Seta 4-C* (Figs. 6,7): The position of seta 4-C in the majority of *Anopheles* larvae is mesad of seta 3-C, usually conspicuously so. In addition to *Kerteszia*, the unusual lateral position of 4-C is known in larvae of *An. kyondawensis* Abraham and the arboricolous *An. sintonoides* Ho (Harrison and Scanlon 1975), two North American arboricolous species, *An. barberi* Coquillett and *An. judithae* Zavortink (Zavortink 1970), and at least two species of the Oriental Aitkenii Group. This list is probably not exhaustive.

Setae 5-7,11-C (Table 1; Figs. 6,7): These four setae are treated together because they have similar morphological characteristics and ontogenetic implications. We have included example illustrations of heads of first

and fourth instar larvae of the genus *Bironella* and three subgenera of *Anopheles* in order to illustrate the course of development of these setae. Figure 6 shows the very striking difference in the development of these setae in fourth instars of *Kerteszia* and *Nyssorhynchus*. Figures 6 and 7 show the difference in the development of these setae in the first and fourth instars of *Bironella* (*Brugella*) and the *Anopheles* subgenera *Anopheles*, *Cellia* and *Nyssorhynchus*. The plumose condition of these setae in fourth instars is found in the majority of *Anopheles*. A first instar of *Kerteszia* was not available, but it is almost certain that these setae are each simple, suggesting a stasimorphic condition in the fourth instar. On the other hand, two different conditions occur between first and fourth instars of most other anophelines. All instars of the genus *Bironella* and several species of the subgenus *Anopheles* have these setae fully developed, i.e., "plumose." In *Bironella* and the subgenus *Anopheles*, the development of these setae in first instars is a clear case of heterochrony (acceleration) where the expression of the plumose condition is well advanced (relative to the expected non-plumose condition in the ontogeny of ancestral forms). In *Nyssorhynchus* (Fig. 6; also Galvão and Lane 1936) and the subgenus *Cellia*, the plumose condition is not expressed in first instars (ancestral condition). It has often been suggested that *Bironella* is the most primitive genus of the Anophelinae, *Anopheles* is the most primitive subgenus of the genus *Anopheles*, and that *Cellia* is the most derived subgenus of the genus *Anopheles*. We do not wish to speculate on the meaning of the above, but we are confident that these setal characteristics will play a significant part in any future phylogenetic analysis of the Anophelinae.

Seta 14-III (Fig. 8): The absence of this seta in larvae of *Kerteszia* was mentioned above under seta 14-III of the pupa. As far as we know, seta 14-III is present in larvae of other groups of *Anopheles*.

Seta 4-X (Fig. 9): The common number of pairs of individual setae in the group designated 4-X (ventral brush) in *Anopheles* is nine. Only eight pairs occur in *Nyssorhynchus* and the genus *Bironella*. We believe this is a

significant character difference with possible phylogenetic implications.

Systematics. The typological definition of species makes use of stable, distinctive morphological characters that are not necessarily adaptive. This definition is most useful in classification. In any analysis of classification, it is essential to distinguish genetically (phylogenetically) fixed characters from those which are environmentally induced. The question here is whether the morphological differences observed between species of *Kerteszia* and *Nyssorhynchus* are the result of independent evolutionary descent or merely environmentally induced variations of labile characters. To answer this question, it is necessary to consider the temporal scale of biological response to environmental adaptation. The first step involves direct effects on the metabolism of individual organisms, i.e., physiological adjustments or responses of an organism which favor survival in a changed or new environment. The next step involves biological responses that occur over many generations, the period for selection of genetic variants. Secondary morphological changes often accompany these responses. Identification of genetically fixed morphological differences between populations and related species living in slightly different niches or ranges is the basis for phylogenetic correlation. These are the variations associated with speciation. Recently evolved sister species or species groups usually differ only slightly in overall morphology. But what about the fact that groups of varying species show very pronounced differences from other groups of varying species, e.g., *Kerteszia* and *Nyssorhynchus*? Is the magnitude of differences indicative of evolutionary change or simply the establishment of secondary characters associated with environmental requirements, in this case the fixation of characters associated with the bromelicolous habit of *Kerteszia*? It appears that the degree of difference is much less important than the constancy of difference, i.e., the discontinuity between the groups. Whether the discontinuity between these groups is the result of slow evolution or a quantum event is also unimportant. The basic fact is that evolutionary changes are

influenced by biotic factors, so the question becomes: are the observed differences indicative of differentiation at the subgeneric level or differentiation at the species group level within the subgenus? This question is answered by comparative analysis of homologous characters in the different subgenera of *Anopheles*. As we have shown, this analysis yields a preponderance of evidence for the subgeneric (perhaps generic) status of *Kerteszia*, and dispels the idea that the included species are merely specialized members of the subgenus *Nyssorhynchus*.

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